World Journal of Microbiology & Biotechnology (2005) 21:1499–1507
DOI 10.1007/s11274-005-7370-7

Selection and optimization of culture medium for exopolysaccharide production by *Coriolus* (*Trametes*) *versicolor*

A.P.M. Tavares[1], M.S.M. Agapito[1], M.A.Z. Coelho[2], J.A. Lopes da Silva[1], A. Barros-Timmons[1], J.A.P. Coutinho[1] and A.M.R.B. Xavier[1,*]
[1]*QOPNA; CICECO – Departamento de Química, Universidade de Aveiro, 3810-193, Aveiro, Portugal*
[2]*Departamento de Engenharia Bioquímica, EQ/UFRJ, Bloco E, Lab.113, 21949-900, Rio de Janeiro, Brasil*
**Author for correspondence: Tel.: +351-234370716, Fax: +351-234370084, E-mail: abx@dq.ua.pt*

Received 9 March 2005; accepted 13 May 2005

Keywords: *Coriolus versicolor*, exopolysaccharide production, experimental design, optimization, submerged culture

Summary

Coriolus versicolor is a medicinal fungus producing exopolysaccharides (EPS). Five well-defined culture media were studied to select the medium that maximizes production of EPS by *C. versicolor*. Biomass, reducing sugars and EPS concentrations along with the rheological behaviour of the broth were followed during fermentations lasting 9 days. The yeast malt extract medium (YM) was shown to yield the highest production of EPS. Fermentation conditions with YM medium were further investigated to optimize EPS production by *C. versicolor*. An experimental design to do this was adopted, in which the effects of pH and initial substrate concentration were considered. The effects of initial glucose concentration (5, 15 and 25 g l^{-1}) and pH (4.0, 5.5 and 7.0) were evaluated. The initial glucose concentration was found to be the most important factor in EPS production and also cell growth.

Introduction

Exopolysaccharides (EPS) from microorganisms have been extensively studied due to the wide range of applications in various fields, including the food, pharmaceutical and cosmetics industries (Mansel 1994; Vuyst 2000) and their medicinal properties (Lee *et al.* 1990; Kuo *et al.* 1996). Studies on polysaccharides with hypolipidaemic effect (Yang *et al.* 2002), antitumour activity (Sugiura *et al.* 1980), immunostimulating activity (Kuo *et al.* 1996) and hypoglycaemic activity (Kiho *et al.* 1993, 1997) have been reported. Most polysaccharides produced by fungi possess biological and pharmacological activities. Various fungi have been described to produce this type of EPS, such as *Paecilomyces japonica* (Bae *et al.* 2000; Sinha *et al.* 2001a), *Cordyceps militaris* (Park *et al.* 2002), *Tremella* species (Khondkar *et al.* 2002), *Auricularia polytricha* (Yang *et al.* 2002) among others.

C. versicolor, belonging to the Basidiomycetes class, can produce both extracellular and intracellular polysaccharides that have received special attention due to their physiological and biological activity. These fungi are well known as a medicinal mushroom in traditional therapeutic practice in Japan, China, Korea and other Asian countries (Cui & Chisti 2003). Their polysaccharides have shown antitumour activity and include protein-bound polysaccharides extracted from the fungal mycelium like the Krestin (PSK) and Polysaccharopeptide (PSP) (Sugiura *et al.* 1980; Ng 1998) and the extracellular polysaccharide Coriolan (Miyazaki *et al.* 1974).

Most of the reported studies have focused on polysaccharides isolated from the mycelium. However, a few studies on EPSs from *C. versicolor* in submerged culture have been reported (Kim *et al.* 2002). Although a number of works have attempted to obtain the best culture conditions and EPS characterization from different fungi, the effect of medium composition on *C. versicolor* fermentations and cultivation kinetics, which are important parameters to EPS production, remain relatively unexplored.

Response surfaces, based on experimental designs, have been used in several fields of bioprocesses and it has been demonstrated to be an adequate tool to evaluate the effects and interactions of the different parameters that rule a biochemical system (Box *et al.* 1978). However, so far experimental designs have not been applied to EPS production by *C. versicolor*.

The aim of this study was to define experimental conditions to optimize EPS production by *C. versicolor*. Firstly a fermentation broth was selected and after that an experimental design and response surface methodology was applied to optimize the medium and the culture conditions.

Materials and methods

Microorganism

C. versicolor was kindly provided by National Institute of Industrial Engineering and Technology (INETI, Portugal). The stock culture was maintained on Petri dish with Tien and Kirk medium (Tien & Kirk 1988), stored at 4 °C and transferred monthly.

Inoculum preparation

C. versicolor was grown in Petri dishes during 7 days at 28 °C (Tien & Kirk 1988). To obtain the inoculum, a mycelial suspension was prepared from Petri dish plates. 10 ml of specific liquid culture medium were added to each Petri dish. With a sterilized wire loop, the mycelium was collected and suspended on liquid medium from where it was transferred to a flask to obtain a concentrated suspension of cells, the inoculum. A given volume of suspension was filtered though 0.45 μm fibreglass filters (GF/C, Watman) and a fast determination of biomass concentration by dry weight was carried using an infrared lamp for 2 h to dry the sample. This allows the calculation of the volume of inoculum necessary to obtain an initial cell concentration of 70 mg l^{-1}. The inoculum was transferred to each 500-ml Erlenmeyer flasks, with 250 ml of culture medium.

Culture media

Five media were used to evaluate the optimum EPS production.

(1) *Trametes defined medium* (TDM): (Roy & Archibald 1993). Glucose 9 g l^{-1}, glutamine 0.78 g l^{-1}; NaCl 0.28 g l^{-1}; KH_2PO_4 0.68 g l^{-1}; $MgSO_4{\cdot}7H_2O$ 0.25 g l^{-1}; thiamine 0.81 mg l^{-1} and 1 ml of element solution.

Element solution: $FeSO_4{\cdot}7H_2O$ 20 μM; $CuSO_4{\cdot}5H_2O$ 2 μM; $ZnCl_2$ 5 μM; $MnSO_4{\cdot}H_2O$ 20 μM; $CoCl_2{\cdot}6H_2O$ 6 μM; $NiCl_2{\cdot}6H_2O$ 0.1 μM and $(NH_4)_6MO_7O_{24}{\cdot}4H_2O$ 0.5 μM.

(2) *Modified Tien & Kirk medium* (TaK) (Tien & Kirk 1988): glucose 10 g l^{-1}; malt extract 10 g l^{-1}; peptone 2 g l^{-1}; yeast extract 2 g l^{-1}; asparagine 1 g l^{-1}; KH_2PO_4 2 g l^{-1}; $MgSO_4{\cdot}7H_2O$, 1 g l^{-1}; thiamine 1 mg l^{-1} but without the 20 g l^{-1} of agar.

(3) *Yeast malt extract medium* (YM) (Kim *et al.* 2002): glucose 10g l^{-1}; malt extract 3 g l^{-1}; peptone 5 g l^{-1}; yeast extract 5 g l^{-1}.

(4) *Mushroom complete medium* (MCM) (Kim *et al.* 2002): glucose 20 g l^{-1}; KH_2PO_4 0.46 g l^{-1}; K_2HPO_4 1 g l^{-1}; $MgSO_4{\cdot}7H_2O$ 0.5 g l^{-1}; peptone 2 g l^{-1}; yeast extract 2 g l^{-1}.

(5) *Glucose yeast extract peptone* (GPY) (Khondkar *et al.* 2002): glucose 40 g l^{-1}; peptone 10 g l^{-1}; yeast extract 3 g l^{-1}.

Shake flask cultures

Each culture medium was inoculated with an initial cell concentration of 70 mg l^{-1} and cultivated for 9 days at 28 °C using an orbital shaker at 180 rev/min. The liquid cultures were carried out in 500-ml Erlenmeyer flasks containing 250 ml of medium. The media were previously sterilized at 121 °C for 21 min.

Optimization of best medium by experimental design

In order to maximize the EPS production, a Full Factorial Design for two independent variables was adopted. The experimental design was based on Statistica 5.0 (Statsoft Inc.). The cultivation conditions were the same as described in shake flask cultures. Full Factorial Design was used to obtain the combination of values that can optimize the response within the region of the three dimensional observation spaces, which allows one to design a minimal number of experimental runs (Box *et al.* 1978). The variables, initial glucose concentration and initial pH, were submitted for the analysis in the design. The variables were chosen for being the most significant variables for enzyme production by this fungus (Tavares *et al.* 2005). The variable of each constituent at levels −1, 0 and +1 is given in Table 1. The selection of low, middle and high levels for all these variables were based on a prior screening done in our laboratory. A 2^2 full factorial design with a central point and an additional experiment with initial glucose concentration of 15 g l^{-1} and pH of 4.0 lead the total number of 6 experiments. The response was the maximum EPS concentration attained during the production. The behaviour of the present system is described by Equation (1), where the model included linear/quadratic main effects only:

$$\hat{Y}\hat{y} = \beta_0 + \beta_1 x_G + \beta_2 x_{\mathrm{pH}} + \beta_3 x_{G^2} + \beta_4 x_{\mathrm{pH}}^2 \qquad (1)$$

where $\hat{Y}\hat{y}$ is the predicted response, *i.e.* the EPS production; x_G, x_{pH} are the independent variables and the regression coefficients were: β_0 the intercept term; β_1, β_2 coefficients for linear effects, β_3, β_4 coefficients for second order interaction.

EPS recovery

To isolate the EPS from the culture medium, the cells were removed in a centrifuge (Sigma 3K30) operated at 11,700 × *g* and 4 °C for 20 min. The supernatant was added to four times its volume of ethanol, stirred and left overnight at 4 °C. The precipitated EPS was

Table 1. Factor levels for a 2^2 factorial design.

Variable	Parameter	Coded level		
		+1	0	−1
G	Glucose (g l^{-1})	25	15	5
pH	Initial pH	4	5.5	7

centrifuged at 11,700 × *g* and 4 °C for 20 min and the supernatant was discarded. This precipitated EPS was sequentially washed with 60, 70, 80, 90 and 100% (v/v) ethanol/H_2O, lyophilized and weighted.

Analytical methods

The biomass concentration was determined by dry weight of fungal mycelium. The culture medium was centrifuged in a centrifuge (Sigma 3K30) operated at 11,700 × g and 4 °C for 20 min. The cells were washed with distilled water at 50 °C under constant stirring and centrifuged again at the same conditions. The supernatant was discarded and the biomass was dried at 100 °C to constant weight.

Culture broth flow behaviour was followed by steady–shear tests using a controlled stress rheometer and a cone-and-plate measuring system (cone angle 2°, diameter 6 cm).

Reducing sugars quantification was made using the 3,5-dinitrosalicylic acid (DNS) methodology (Miller 1959).

The relative amounts of monosaccharides were determined by gas–liquid chromatography after hydrolysis with sulphuric acid, and derivatization to alditol acetates (Blakeney *et al.* 1983). Derivatized samples were analysed in a Carlo Erba 6000 gas chromatograph equipped with a flame ionization detector (FID), and a DB-225 column.

The thermogravimetric analysis (TGA) of EPS was carried out using a TGA-50 (Shmadzu) thermal analyser under N_2 atmosphere. A heating rate of 10 °C/min from 20 to 700 °C was employed to investigate the thermal behaviour of the EPS.

GPC-analysis of EPS dissolved in *N,N*-dimethylacetamide (DMCA) containing 0.5% w/v LiCl, was performed on two Plgel 10 μm MIXED B 300 × 7.5 mm columns protected by a Plgel 10 μm Pre-column (Polymer laboratories Ltd. UK) using a PL-GPC 110 system (Polymer laboratories Ltd. UK). The columns, guard columns and injection system were maintained at 70 °C. The eluent (0.5% w/v LiCl in DMAC) was pumped at flow rate 0.9 ml/min. The GPC columns were calibrated using pullulan (Polymer laboratories) reference materials. This analysis was employed to investigate the molecular weight of the EPS from YM medium.

Results and discussion

Selection of culture media

Production of EPS by *C. versicolor* in submerged culture was studied in five different culture media. Tien and Kirk (TaK) is the medium reported for fungus maintenance. Trametes Defined Medium (TDM) is a specific medium referred for enzymatic production by *C. versicolor*. Yeast malt extract medium (YM) as well as Mushroom complete medium (MCM) and Glucose Yeast Extract Peptone (GPY) have been recently reported for polysaccharide production. YM was reported to be the best medium for extracellular polysaccharide production for *Tremella* species, in terms of polymer yield, both for solid and liquid culture when compared with GPY medium and potato dextrose agar medium (PDA) (Khondkar *et al.* 2002). When TDM or GPY media were used in *C. versicolor* cultivations, no production of EPS was observed. TDM being a synthetic defined medium probably fails to provide nutrients to synthesize the EPS required by the fungus. Concerning the GPY medium, the high sugar concentration (40 g l^{-1}) is probably inhibitory for this fungus. The other three media used yielded a reasonable production of EPS.

Figure 1 shows the time courses of biomass (1a), reducing sugars (1b) and EPS production (1c) by *C. versicolor* fermentation in MCM, TaK, and YM media. The results show that the concentration of reducing sugars decreases during the fermentation with a corresponding increase of biomass. The biomass curve (Figure 1a), shows that exponential growth lasts only 2 days. From day 2 to day 4 there is a linear growth

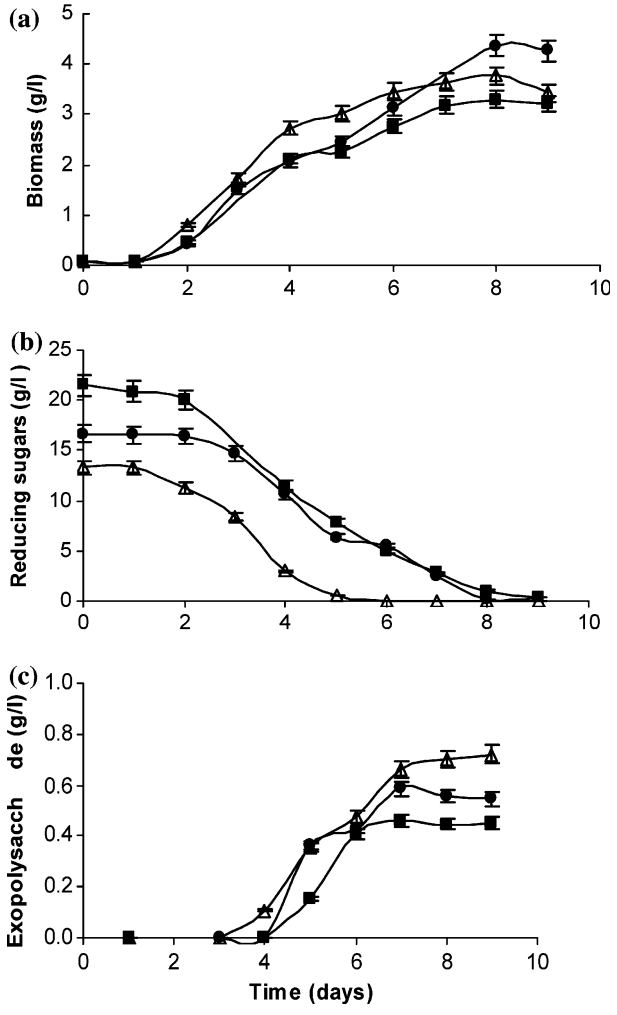

Figure 1. The time courses of biomass (a), reducing sugars (b), EPS production (c) during the fermentation of *Coriolus versicolor*: (■) MCM medium; (●) TaK medium; (◇) YM medium.

phase and a deceleration growth phase between days 4 and 7 of cultivation. Tavares *et al.* (2005) recently reported these two phases for this fungus under other experimental conditions. The EPS production starts only at day 4, showing that production of this polysaccharide is not associated with exponential growth. The EPS production appears when active cell growth has ceased and the cells begin the deceleration growth phase. This behaviour is typical of products that are not produced by the primary metabolism (trophophase), which is essentially associated to exponential growth, but by a secondary metabolism. Usually secondary metabolites are produced at the beginning of the deceleration phase (idiophase) (Waites *et al.* 2001).

Comparing the EPS production on the three culture media in Figure 1c, it can be seen that the EPS production essentially stopped after day 7 for all media. It attained a maximum concentration of 700 mg l^{-1} (Table 2) on the last day of fermentation for the YM medium while for TaK and MCM media the maximum EPS concentration obtained was of 590 and 460 mg l^{-1}, respectively. It should be noticed that for both media the EPS production finished before total substrate consumption. The EPS production obtained with YM in the present study (700 mg l^{-1}) is higher than reported by Kim *et al.* (2002) for *C. versicolor* whose maximum exobiopolymer production in YM medium was 504 mg l^{-1}.

Table 2 summarizes cultivation data of EPS production by *C. versicolor* on TaK, MCM, and YM media. Although the highest biomass concentration and yield (g of biomass/g of consumed reducing sugars) were obtained with TaK medium, the maximum EPS concentration and productivity was achieved using YM medium, as described above. Calculating the maximum EPS/biomass (g/g) ratio it is clear that YM was the medium that provided the best results, 0.24 g/g, which is 1.5 and 1.7 times higher when compared with TaK and MCM, respectively. On the other hand TaK was the best medium for biomass production with final concentrations of 4.4 g l^{-1} whilst YM and MCM attained only 3.8 and 3.3 g l^{-1}, respectively.

Table 2. Data from *Coriolus versicolor* cultivation on different culture media.

Cultivation parameters	Culture medium		
	TaK	MCM	YM
Maximum biomass concentration (g l^{-1})	4.4	3.3	3.8
Maximum EPS concentration (mg l^{-1})	590	460	700
Maximum apparent viscosity (mPa.s)	26.0	16.0	47.1
Maximum EPS productivity (g l^{-1} day^{-1})	0.073	0.068	0.094
Yield (g of biomass/g of reducing sugars consumed)	0.24	0.17	0.23
Maximum EPS/biomass (g/g)	0.16	0.14	0.24

Optimization of EPS production in YM medium by experimental design

The results presented above indicate that YM was the best culture medium for EPS production and it was selected for further optimization. It is known that glucose as carbon source and pH as physical factor are two major culture conditions that can provide both optimal cell growth and product formation. *C. versicolor* seems to be particularly sensitive to these two parameters as shown before for enzyme production (Tavares *et al.* 2005). To select the optimal pH and glucose concentration for EPS production, *C. versicolor* cultivation was studied on YM media covering a range of values for these parameters. To find the best experimental conditions a factorial design with two levels was adopted. Results of *C. versicolor* fermentations used in the factorial design are summarized in Table 3. The analysis shows that the media with glucose of 15 g l^{-1} and pH of 5.5 promoted the best production and productivity of EPS and cell growth. These results show that this initial glucose concentration was favourable to the EPS production due to a relatively high cell density obtained under these conditions. Higher initial glucose concentration did not stimulate the EPS formation, resulting in production lower than 300 mg l^{-1} and also the biomass growth.

Individually calculated effects and their interactions for the 2^2 factorial design are reported in Table 4. The variable with largest effect on EPS production was the glucose concentration. The linear effect (L) of glucose concentration (G) was to reduce the EPS production by about 93 units when an increase of 5–25 g l^{-1} was employed. The significance of each coefficient was determined through a P-value test ($P < 0.1$), considering 90% of confidence, where low P-values indicate high significance of the corresponding coefficient (Table 4).

Factorial design results could be represented through a surface, called response surface. The response surface was used to optimize the EPS production. The resulting response surface shows the effect of initial glucose concentration and pH on EPS production (Figure 2). The Figure 2 represents a three-dimensional plot of the

Table 3. 2^2 factorial design and responses for exopolysaccharide production by *Coriolus versicolor*.

Run	Initial Glucose (g l^{-1})	Initial pH	Final biomass (g l^{-1})	Maximum EPS production (mg l^{-1})	EPS Productivity (mg /(l.day))
F1	5	4	3.0	380	47.5
F2	5	7	2.7	380	47.5
F3	15	5.5	4.2	640	80.0
F4	15	4	3.8	520	74.3
F5	25	4	2.5	274	34.25
F6	25	7	2.3	300	37.5

Table 4. Individually calculated effects for the 2^2 factorial design.

Effects	Estimate	*t*-value	*P*-value	SS	Df	MS	*F*
Glucose (L)	−93.0	−7.1538	0.0884	8649.00	1	8649.00	51.1775
Glucose (Q)	−386.0	−12.1218	0.0524	24832.67	1	24832.67	146.9389
PH (L)	13.0	1.0000	0.500	169.00	1	169.00	1.0000
PH (Q)	−227.0	−5.8205	0.1083	5725.44	1	5725.44	33.8784
Error				169.00	1	169.00	
Total SS				97203.33	5		

$R^2 = 0.99826$.

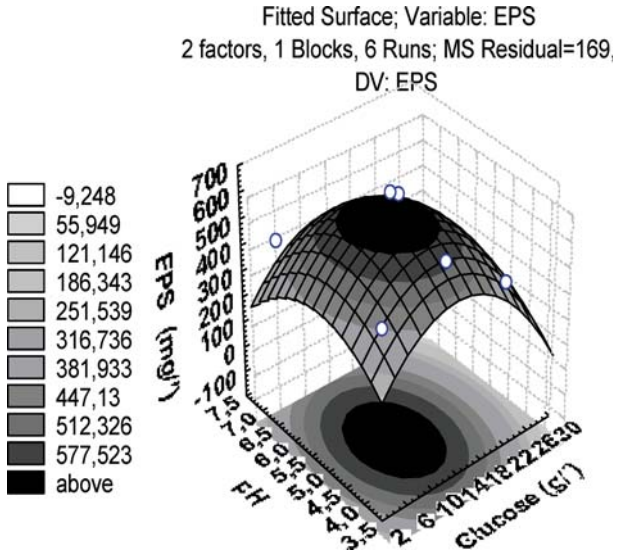

Figure 2. Response surface for the effect of initial glucose concentration and pH on Exopolysaccharide production by *Coriolus versicolor*.

following variables: glucose concentration and initial pH. The maximum predicted value of EPS production was 642.9 mg l^{-1} at glucose concentration of 13.8 g l^{-1} and pH of 5.5.

In this study the model used contains only linear and quadratic main effects. The model containing linear and quadratic main effects and two-way interaction effect was inadequate to represent the experimental data. The Equation 2 was obtained from data regression analysis of experiment design:

$$\hat{Y}\hat{y} = -1274.28 + 53.25 \times G - 1.93 \times G^2 + 559.22 \times \mathrm{pH} - 50.44 \times \mathrm{pH}^2 \quad (2)$$

For higher substrate concentration (above 20 g l^{-1}), the metabolism of EPS biosynthesis by *C. versicolor* and the cell growth is probably inhibited. Fang & Zhong (2002) suggested that in the presence of higher glucose concentration (above 50 g l^{-1}) the growth of the fungus *Ganoderma lucidum* and intracellular polysaccharide production were inhibited due the unfavorable osmotic pressure, however the high glucose concentration led to high extracellular polysaccharide production. The production of EPS by the fungus *Paecilomyces japonica* also showed that the concentration of carbon source, an important factor to biopolymer production and fungal growth, also influences the fungal morphology and broth rheology, which alters the transport characteristics in high concentrations (above 40 g l^{-1}) (Sinha *et al.* 2001b).

Biopolymer characterization: sugar profiles and chemical composition

Neutral sugars from the EPS produced in the different media were analysed using the Blakeney *et al.* (1983) methodology and the results are summarized in Table 5. The predominant sugar in these EPS is glucose, with a relative amount of 98%, 95% and 88% when produced in YM medium, TaK medium and MCM medium, respectively. Smaller amounts of mannose and xylose were also determined in the EPS produced in these three media with different percentages. The EPS produced in the MCM medium contains 3% of other sugars, which include rhamnose, arabinose, galactose and fucose. These results indicate that the culture medium influences the neutral sugar composition of the EPS produced by *C. versicolor*. However, regardless of the medium, the predominant component is glucose, which is in agreement with data previously published for this fungus by

Table 5. Relative proportions of monosaccharides from EPS produced by *Coriolus versicolor* in yeast malt extract medium, mushroom complete medium and Tien and Kirk medium.

Media	Neutral sugars (%, w/w)			
	Glucose	Mannose	Xylose	Others(*)
Mushroom complete medium	88%	7.4%	1.6%	3%
Yeast malt extract medium	98%	1.2%	0.8%	traces (§)
Tien and Kirk	95%	3.4%	1.6%	traces (§)

(*) Rhamnose, Arabinose, Galactose, Fucose.
(§) ≤0.1%.

Miayazaki *et al.* (1974) who estimated a 97.2% content of glucose for the EPS obtained using an onion- and soya-based medium (Ng 1998) and also by Sugira *et al.* (1980) with the sugar composition reported for the EPS extracted from the mycelium of this fungus.

Thermal gravimetric analysis (TGA) of this polysaccharide was also carried out. As can be observed in Figure 3 the EPS is thermally stable up to 280 °C. Above this temperature decomposition takes place in two stages: between 280 °C and 350 °C a fast weight loss is observed i.e. ca. 60% of the weight loss of the polysaccharide occurs. This is followed by a second decomposition stage from 350 to 560 °C, during which a more gradual decomposition is registered possibly due to the decomposition of polysaccharide aggregates. No further weight loss is registered up to 700 °C indicating a 7.5% of inorganic residue. The profile of the TGA curve is consistent with those obtained for various polysaccharides from microbial origin characterized in our laboratory.

The average molecular weight distribution of the EPS obtained on the YM medium by GPC analysis was approximately 67 kDa, which compares with the values for the PSK and PSP polysaccharide of *C. versicolor* that have a molar mass of approximately 100 kDa (Cui & Chisti 2003). The gel permeation chromatogram is shown in Figure 4, where the polydispersity of the EPS is presented. The main elution peak appears between 12 and 18 min.

Broth rheology

Qualitatively, the flow rheological behaviour of the different media is similar (Figure 5). During the initial 4-day period, before the beginning of significant EPS production, the flow behaviour is essentially Newtonian. After that the behaviour changes to the typical shear-thinning behaviour expected for aqueous polysaccharide dispersions.

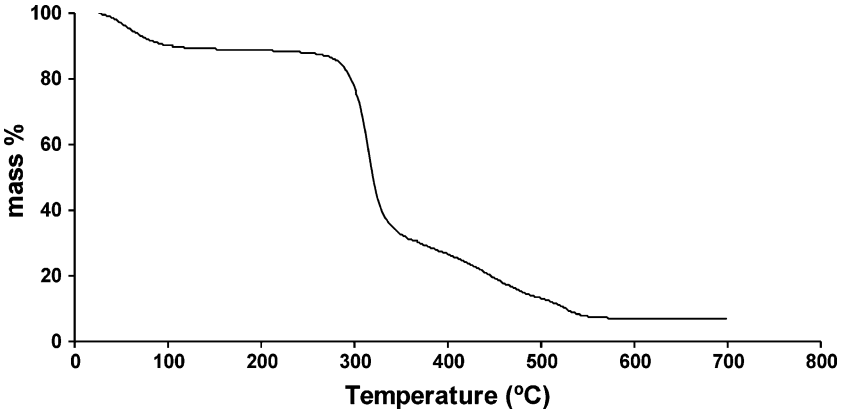

Figure 3. Thermogravimetric analyses (TGA) of the EPS produced by *Coriolus versicolor* in YM medium.

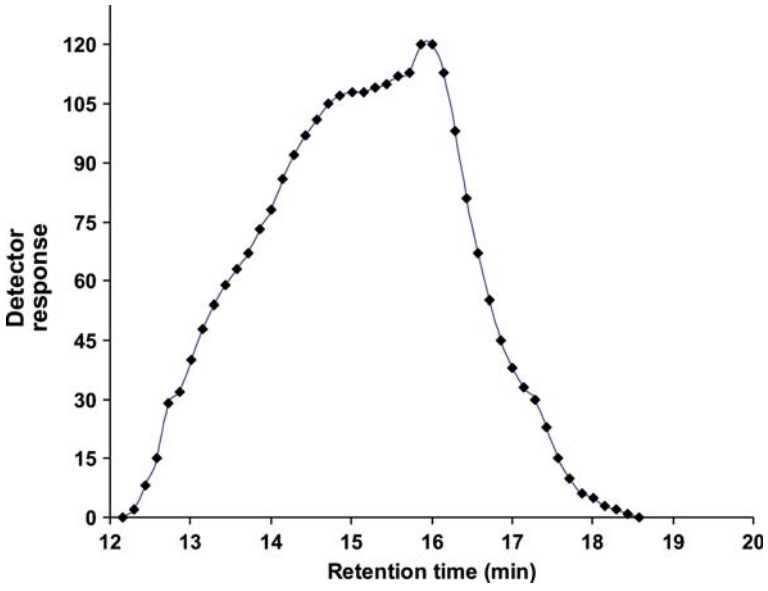

Figure 4. GPC elution curve of EPS obtained by *Coriolus versicolor* in YM medium.

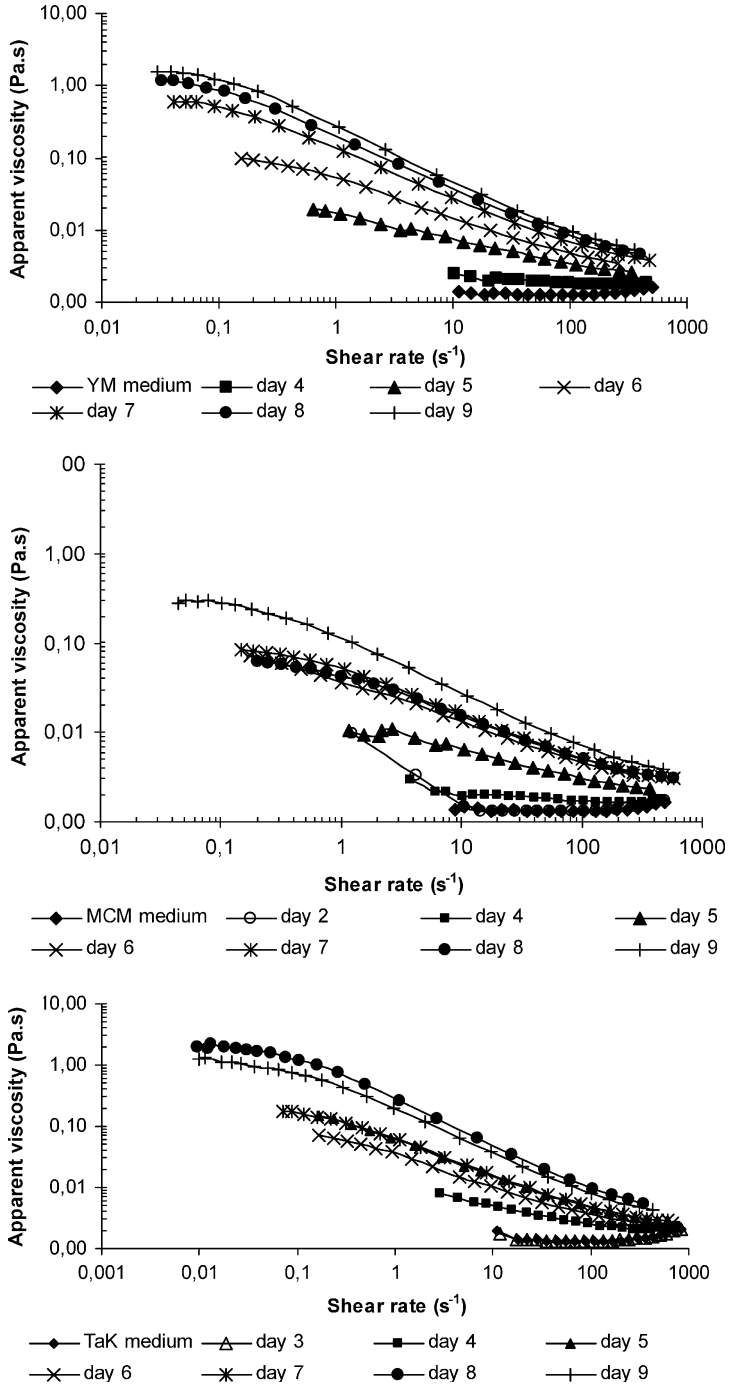

Figure 5. Apparent viscosity of *Coriolus versicolor* cultivation broth to: (a) YM medium; (b) MCM medium; (c) TaK medium.

Data from Figure 6 were used to calculate apparent viscosity (shear rate of 10 s^{-1}) for the entire cultivation period in these three different media. The results show that the apparent viscosities of the cultivation broth increased rapidly from day 4 of cultivation. For the YM medium the apparent viscosity increased continuously until day 8, in accordance with the results obtained by EPS dry weight. For the MCM and TaK the apparent viscosity increased until days 6 and 7, respectively and the viscosity remained constant until the end of cultivation for MCM, whilst in the case of the TaK medium a small decrease was observed.

The pseudoplastic behaviour is characteristic of polymers in solution (Whistler *et al.* 1997) being the degree of pseudoplasticity of a polymer solution dependent on the polymer concentration. Comparing Figures 1c and 6, a good agreement can be noticed between polymer concentration and cultivation broth viscosity. Since polymer quantification by dry weight is a tedious methodology, the evaluation of polymer production during cultivation time can be done in a fast and accurate way by following the broth rheology. A well established logarithmical correlation between EPS concentration and broth viscosity was used that can be represented by Equation 3 ($R^2 = 0.9903$):

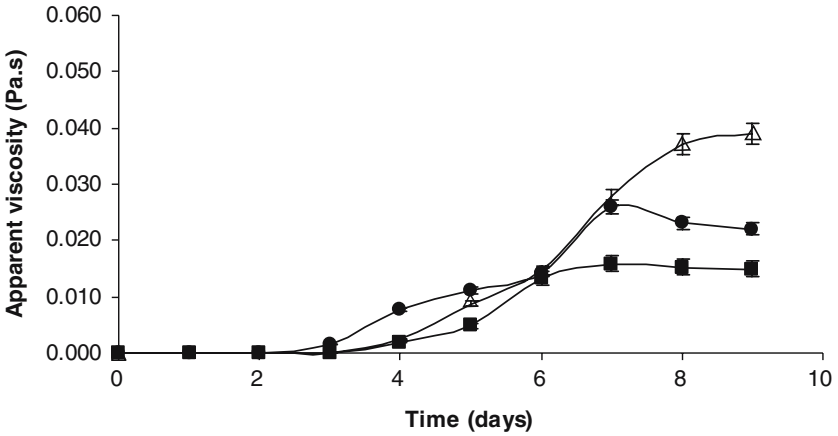

Figure 6. Apparent viscosity (shear rate of 10 s^{-1}) of the fermentation broth of *Coriolus versicolor* in different media, as a function of fermentation time: (■) MCM medium; (●) TaK medium; (◇) YM medium.

$$Y = 235.64 \ln(X) + 1495.5 \quad (3)$$

where X is the EPS concentration (mg l^{-1}) and Y is the Apparent Viscosity (Pa.s).

A rapid determination of EPS concentration of *C. versicolor* could be obtained through rheological analysis of the culture medium.

Conclusions

It was shown that the culture medium plays a major role in EPS production and fungal growth by *C. versicolor*. YM is the best medium, among the media studied, to produce EPS. It seems that high sugar concentrations (40 g l^{-1}) are inhibitory to *C. versicolor* growth (GPY) and that the chemically defined media (MDT) used for ligninolytic enzymatic production is not adequate to develop EPS production. The other media tested (YM), (MCM) and (TaK) have in common the yeast extract and peptone. The higher concentrations of yeast extract in the YM medium seem important for EPS production. The rheology of the fermentation broth for these three media is qualitatively very similar. In this work, broth rheology has been proved to be a good and fast analytical methodology for following EPS production. The methodology of experimental factorial design and response surface analysis permit determination of the optimal initial glucose concentration and pH to obtain EPS production by *Coriolus versicolor*.

Acknowledgements

The authors acknowledge the Instituto Nacional de Engenharia e Tecnologia Industrial (INETI) for *Coriolus versicolor* cultures and CAPES/GRICES (102/03). A.P.M. Tavares thanks Fundação para Ciência e Tecnologia (FCT) for a PhD grant (SFRH/BD/6606/2001) and M.A.Z. Coelho thanks CNPq (Conselho Nacional de Desenvolvimento Cientifico e Tecnológico, Brazil).

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